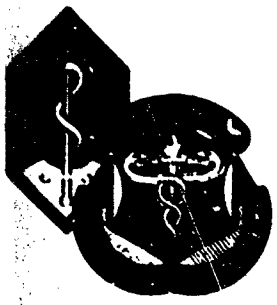


INSTITUTE REPORT NO. 174

ACUTE DERMAL TOXICITY POTENTIAL OF THE HOLSTON COMPOUNDS: VIRGIN DMSO, DMSO RECYCLE SOLVENT, AND DMSO EVAPORATOR SLUDGE IN MALE AND FEMALE RABBITS

LAWRENCE MULLEN, BS, SP5
CRAIG W. WHITE, DVM, CPT VC
and
GLEN E. MARRS, DVM, MS, MAJ VC

TOXICOLOGY GROUP,
DIVISION OF RESEARCH SUPPORT

JUNE 1984

Toxicology Series 69

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER LAIR Institute Report No. 174	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Acute Dermal Toxicity Potential of the Holston Compounds: Virgin DMSO, DMSO Recycle Solvent, and DMSO Evaporator Sludge in Male and Female Rabbits		5. TYPE OF REPORT & PERIOD COVERED Final 17 Feb 83 - 5 Jul 83 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Lawrence Mullen, BS, SP5 Craig W. White, DVM, CPT VC Glen E. Marrs, DVM, MS, MAJ VC		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS Toxicology Group, Division of Research Support Letterman Army Institute of Research Presidio of San Francisco, CA 94129		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 61270.835AA
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command Fort Detrick Frederick, MD 21701		12. REPORT DATE June 1984 13. NUMBER OF PAGES 48
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) UNCLASSIFIED 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

THIS DOCUMENT HAS BEEN CLEARED FOR PUBLIC RELEASE AND SALE: ITS DISTRIBUTION IS UNLIMITED.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

DMSO Recycle Solvent (TP013), Virgin DMSO (TP014), DMSO Evaporator Sludge (TP015), Acute Dermal Toxicity, Holston Army Ammunition Plant, Nitramines

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The acute dermal toxicity potential of the Holston Compounds (Virgin DMSO, DMSO Recycle Solvent, and DMSO Evaporator Sludge) was determined in rabbits by topical application to skin sites with plastic covering over the exposed areas for 24 hours. There were no compound-related deaths at a limit dose of 2 ml/kg during this study. The Holston Compounds caused minimal dermal irritation. Keywords: explosives; recrystallization; neurotoxins; solvents

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

ABSTRACT

The acute dermal toxicity potential of the Holston Compounds (Virgin DMSO, DMSO Recycle Solvent, and DMSO Evaporator Sludge) was determined in rabbits by topical application to skin sites with plastic covering over the exposed areas for 24 hours. There were no compound related deaths at a limit dose of 2 ml/kg during this study. The Holston Compounds caused minimal dermal irritation.

KEY WORDS: Virgin DMSO, DMSO Recycle Solvent, DMSO Evaporator Sludge, Acute Dermal Toxicity, Holston Army Ammunition Plant, Nitramines

PREFACE

TYPE REPORT: Acute Dermal Toxicity GLP Report

TESTING FACILITY: U.S. Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

SPONSOR: U.S. Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94192

PROJECT/WORK UNIT/APC: DMSO Recrystallization Solution
612720.835AA, APC TL06

GLP STUDY NUMBER: 82038

STUDY DIRECTOR: COL John T. Fruin, DVM, PhD, VC
Diplomate, American College of
Veterinary Preventive Medicine

PRINCIPAL INVESTIGATOR: CPT Craig White, DVM, VC

CO-PRINCIPAL INVESTIGATOR: SP5 Lawrence Mullen, BS

PATHOLOGIST: MAJ Glen E. Marrs Jr., DVM, MS, VC
Diplomate, American College of
Veterinary Pathologists

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocol, retired SOPs, raw data, analytical, stability, and purity data of the test compound, tissues, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCES: The Holston Compounds
a. DMSO Recycle Solvent (TP013)
b. Virgin DMSO (TP014)
c. DMSO Evaporator Sludge (TP015)
d. Saline Control

INCLUSIVE STUDY DATES: 17 Feb 83 - 5 Jul 83

OBJECTIVE: The purpose of this study was to determine the acute dermal toxicity potential of the Holston Compounds in rabbits.

ACKNOWLEDGEMENTS

The authors wish to thank SP5 Leonard Sauers, MS; SP5 Florence McKinley, BS; SP5 Marlin McKinley, BS; SP5 Thomas Kellner, BA; SP5 Justo Rodriguez, BS; SP5 Evelyn Zimmerman; Carolyn Lewis, MS; Thomas Hironaga; Lucille Cote; and John Dacey for their assistance in performing the research. In addition, we wish to thank Jesse Barkley Jr., US Army Medical Bioengineering and Development Laboratory, for his assistance as Project Consultant.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY:

We, the undersigned, believe the study number 82038 described in this report to be scientifically sound and the results in this report and interpretation to be valid. The study was conducted to comply, to the best of our ability, with the Good Laboratory Practice Regulations for Non-Clinical Laboratory Studies, outlined by the Food and Drug Administration.

JOHN T. FROIN / DATE
COL, VC
Study Director

LAWRENCE MULLEN / DATE
SP4, USA
Co-Principal Investigator

GLEN E. MARRS / DATE
MAJ, VC
Pathologist

CRAIG W. WHITE / DATE
CPT, VC
Principal Investigator

DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA 2 May 84

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 82038 following inspections were made:

21 Mar 83

21 Jun 83

1 Jul 83

The report and raw data for this study were audited on 12 Apr 84.

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the 29 Nov 83 report to Management and the Study Director.

NELSON R. POWERS, Ph.D.
DAC
Chief, Quality Assurance Unit

TABLE OF CONTENTS

Page

Abstract.....i

Preface.....iii

Acknowledgments.....iv

Signatures of Principal Scientists.....v

Report of Quality Assurance Unit.....vi

Table of Contents.....vii

BODY OF REPORT

INTRODUCTION.....1

Toxicity Testing.....1
Description of Test.....2
Objective of Study.....2

METHODS

Test Substances.....2
Compound Preparation.....3
Animal Data.....3
Husbandry.....3
Group Assignment / Acclimation.....3
Dosing.....4
Observations.....4
Duration of Study.....5
Changes to Original Protocol.....5

RESULTS

Clinical Observations.....6
Treatment of Animal Disease and Injury.....12
Gross Pathological Observations.....12

DISCUSSION.....12

CONCLUSION.....12

RECOMMENDATION.....12

REFERENCES.....13

Table of Contents (continued)

Page

APPENDICES

Appendix A, Chemical Analysis Data.....17
Appendix B, Animal Data.....29
Appendix C, Historical Listing of Study Events.....33
Appendix D, Dermal Irritation Scores.....35
Appendix E, Pathology Report.....41

OFFICIAL DISTRIBUTION LIST.....48

Acute Dermal Toxicity Potential of the Holston Compounds: Virgin DMSO, DMSO Recycle Solvent, and DMSO Evaporator Sludge in Male and Female Rabbits--Mullen et al

The Holston Defense Corporation has proposed that dimethyl sulfoxide (DMSO) be used as the replacement recrystallization process solvent for the synthesis of the explosives hexanydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro- 1,3,5,7-tetrazine (HMX). As a result of this proposal, a pilot recrystallization facility was put into small scale operation. Samples of the DMSO process stream were taken from two locations at the recrystallization facility. The solutions collected were designated DMSO Recycle Solvent and DMSO Evaporator Sludge. The industrial grade DMSO, also sampled, was designated Virgin DMSO. The Process Stream Samples were analyzed by the Holston Defense Corporation Laboratory. Major and minor cyclic and non-cyclic nitramines were found in the samples. Since nitramines are neurotoxic (1), their presence in the samples represented a potential health hazard to workers utilizing this production process. Thus, to delineate the acute toxicity of the DMSO solutions so that a complete health hazard assessment could be obtained is necessary before the DMSO process solvent procedure is put into full scale operation (1-4).

The Toxicology Group of the Letterman Army Institute of Research was designated by the U.S. Army Medical Research and Development Command to perform the initial toxicity testing on the DMSO samples. The initial data will provide a base for further toxicological testing leading to definitive health protection criteria. These criteria will be used to evaluate facility design and worker protection equipment.

Description of Test

Methods of testing compounds for their potential irritancy or toxicity have become standardized over the years by the cooperative efforts of the Environmental Protection Agency (EPA), Food and Drug Administration (FDA), U.S. Consumer Product Safety Commission, numerous subcommittees, and the Armed Forces Research departments (5-7).

A test for acute dermal toxicity is designed to evaluate the potential for systemic toxic effects of chemicals expected to come in contact with the skin. This is done by determining the median lethal dose (LD_{50}) of a single dermal exposure to the animal species under test.

Dermal toxicity is one of the three categories of toxicity defined by route of exposure in the Federal Hazardous Substances Act (FHSA). The adult albino rabbit is the preferred species for such reasons as size, ease of handling and restraint, and because its skin is the most permeable of all species studied. The rabbit appears to be sensitive to dermal insult. The animal's dorsal and lateral sections were close clipped so that no less than 10% of the body surface area was available for application of material (8). The abdominal section was not clipped.

The maximum quantity of test substance applied is 2 ml/kg. The test dose must remain in contact with the skin throughout the 24-hour exposure period. This is assured by application of the dose inside an impermeable cuff made of plastic film. The cuff or sleeve is constructed so that the ends are reinforced and fit snugly around the trunk of the animal. The ends are tucked to permit the central portion to "balloon" and to furnish a reservoir for the dose. Such devices occlude the skin and thereby enhance penetration and potential toxicity of the test material. For this reason, routine use of occlusive dressing is not recommended unless anticipated human exposure warrants it. For materials of anticipated low toxicity, an initial range-finding dose of 2 ml/kg of body weight applied to five or more animals of each sex with abraded skin is sufficient to demonstrate a lack of appreciable dermal toxicity. At the end of the exposure periods, any residual material is gently removed with a gauze compress, the animal is examined at least daily for signs of systemic toxicity and localized dermal reaction. After the 14-day observation period, animals are sacrificied, a gross necropsy performed, and two sections of the exposed skin are processed for histopathology (9).

Objective of Study

The objective of the study is to determine the acute dermal toxicity potential of DMSO recrystallization solvents designated DMSO Recycle Solvent (TP013), Virgin DMSO (TP014), and DMSO Evaporator Sludge (TP015) in rabbits.

METHODS

Test Substances

1. Chemical name: DMSO Recycle Solvent (TP013)
2. Chemical name: Virgin DMSO (TP014)
3. Chemical name: DMSO Evaporator Sludge (TP015)

Identification of nitramine impurities in the test samples by high pressure liquid chromatography (HPLC) was recently performed by the Holston Defense Corporation. Results from these analyses and chemical data on the constituents of the test mixtures appear in Appendix A. Since the samples were three-years-old at the time the study was conducted, no additional analyses were performed at LAIR while the study was in progress. Other information regarding chemical/physical characteristics of the test compound, including stability, are on file with the sponsor.

Isotonic sodium chloride (saline) was used as a control.

Compound Preparation

TP013, TP014, and TP015 were supplied by the Holston Army Ammunition Plant and used in the form provided. No vehicle was used. The recrystallization solvents were placed into a water bath at 40°C before dosing the rabbits and remained in the bath during the dosing procedure. The test materials were applied uniformly over the prepared dorsal surface area (240 cm^2) and held in contact with the skin by a porous gauze dressing for 24 hours.

Animal Data

A total of 44 New Zealand White rabbits (22 males and 22 females) were received from Elkhorn Rabbitry, Watsonville, CA 95076. Additional animal data are found in Appendix B.

Husbandry

All animals in this study were housed one per cage. The cage was a stainess steel battery type with a wire mesh bottom. A commercially avialable certified rabbit chow (Certified Ralston Purina Rabbit Chow 5322) and tap water (central line to cage battery) were provided ad libitum for the animals during the study. All animals had a photoperiod from 0600 to 2000 or 14 hours of light. The temperature was 21 ± 3°C during both the male and female tests. However, the relative humidity ranged from 50% to 78% for the males, and 68% to 80% for the females. During the female testing, relative humidity was 92% on 25 Jun 83 and 90% on 26 Jun 83. This was the weekend selected by in-house engineers to perform maintenance and the entire system was shut down.

Group Assignment / Acclimation

There were four groups with 5 animals of each sex in this study. Animals were randomized manually using a Random Numbers Table. All animals were quarantined for 2 weeks before being placed in the GLP suite.

Dosing

Dose Levels

Originally, the test was to be conducted as a limit test (SOP-OP-STX-30) wherein 5 males and 5 females are assigned to each test chemical TP013, TP014, TP015, and saline control group. However, only male rabbits arrived as scheduled (17 February 1983). The male rabbits, 5 per group, were tested from 7 March 1983 to 4 April 1983. The female rabbits, 5 per group, arrived at LAIR on 2 June 1983 and were tested from 16 June 1983 to 5 July 1983. Each animal in the test and control groups received 2 ml/kg of body weight. If a test is conducted at this dose level and no test compound related mortality occurs, then a full study using 3 dose levels is not necessary (4). For a standard test, 10 animals per dose group would have been used, half of these animals would have the exposed area abraded and the other half would remain intact (10).

Dose Volume (according to weight)

Volumes administered to males ranged from 4.4 to 5.6 ml of test compound and to females ranged from 5.2 to 7.0 ml of test compound.

Duration of Exposure: 24 hours

Method and Frequency of Administration

The application sites in all animals were abraded by use of an abrading tool designed for dermal toxicity studies (11). It has four small metal points mounted onto a flat piece of metal that is attached to a handle which was drawn along the axis of the backbone so that only the integrity of the stratum corneum was disrupted. The test material was administered with a needle-less syringe at the appropriate dose volume. The test material was applied uniformly over the prepared dorsal surface area and held in contact with the skin by a porous gauze dressing. The dorsal and abdominal areas were then covered with plastic wrap (5mm polyethylene) derived from GSA bags (#NSN 8105-00-655-8285) and taped on the ends and seam with Conform^{R} adhesive tape (Kendal Hospital Products, Boston, MA 02110, Code No. 7233). The animals were observed and clinical signs recorded within 2.5 and 5.0 hours after administration of the test material. The bandage was removed after 24 hours. All residue material was removed by washing with saline and then wiping the animals with gauze pads.

Observations

Male rabbits were weighed six times and female rabbits were weighed three times over the study test period. Clinical observations were recorded two times on the day of dosing and once a day for the remainder of the study.

Dermal irritation was recorded according to location, area, and intensity of the lesion and was graded according to a scale located along the edge of the data sheets. This scale includes five indices to define area and severity. Area is defined as ≤5%, ≤10%, ≤25%, ≤50%, and >50% of the close-clipped dorsal section of the rabbit. Severity is defined as very slight, slight, moderate, well-defined, and severe. Examples of dermal irritation include erythema, edema, blister, necrosis, and pitting. Thus an observation would describe erythema as very slight, involving an area ≤10%, and occurring on the back. At the end of the 2-week period, animals were anesthetized with sodium pentobarbital, sacrificed by exsanguination from severed axillary vessels and evaluated at necropsy. Skin was taken from an abraded and non-abraded area and examined microscopically.

Duration of Study

Male Rabbits

The study period was 14 days with a 25-day quarantine/acclimation period.

Female Rabbits

The study period was 14 days with a 19-day quarantine/acclimation period.

Historical study events are listed in Appendix C.

Changes from Original Protocol

The female rabbits did not arrive on schedule.

Male rabbits were underweight when they arrived at LAIR on 17 February 1983. The dose day was postponed until the animals gained the necessary weight.

The male rabbits were reclipped at dosing to assure an adequate exposure to the test compound.

Female rabbits arrived 2 June 1983 previously tattooed. LAIR I.D. numbers were cross referenced IAW SOP-OP-ARG-1.

The day of dosing for female rabbits was changed from 20 June to 21 June 1983.

The dose level was delivered at 2 ml/kg rather than 2 g/kg due to the physical properties of the substances.

Animal 83F345 had to be rewrapped approximately one hour after dosing. The second wrapping was left in place for the required 24-hour period.

These changes to the protocol did not have any adverse effect on the outcome of the study.

RESULTS

Clinical Observations

During the course of the study, observations were split into two major categories, systemic which applied to the general health of the animal and dermal which related to skin exposure. No clinical systemic signs were interpreted as signs of toxicity attributable to the test compounds in either male or female rabbits (Table 1). A summary of clinical observations appears in Tables 2A - 2D. Skin irritation scores for erythema and edema at 24 hours, 48 hours, and 72 hours are presented in Appendix D.

GLP STUDY #82038

TABLE 1

DERMAL TOXICITY POTENTIAL OF HOLSTON COMPOUNDS IN RABBITS

SUMMARY OF ACUTE CLINICAL OBSERVATIONS

(Number of Animals Affected)
(Number of Animals Exposed)

Clinical Signs	DMSO Recycle Solvent		Virgin DMSO		DMSO Evaporator Sludge		Saline Control	
	Males	Females	Males	Females	Males	Females	Males	Females
Death	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Diarrhea	2/5	0/5	1/5	2/5	0/5	1/5	1/5	2/5
Weight Loss	1/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5
Excited	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Incr. Resp. Rate	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Decr. Resp. Rate	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

TABLE 2A

DERMAL TOXICITY POTENTIAL OF MOLSTON COMPOUNDS IN RABBITS

SUMMARY OF ACUTE DERMAL TOXICITY SIGNS

GLP Study #82038 — Group 1 DMSO Recycle Solvent

	Animal Number	Signs of Dermal Irritation	Dates (1983)	Severity (max)	Exposed Area (max)	Location
MALES (N=5)	83F119	None Observed	N/A	N/A	N/A	N/A
	83F120	None Observed	N/A	N/A	N/A	N/A
	83F124	None Observed	N/A	N/A	N/A	N/A
	83F128	None Observed	N/A	N/A	N/A	N/A
	83F134	None Observed	N/A	N/A	N/A	N/A
FEMALES (N=5)	83F335	Erythema	27 Jun	SL	10	B
		Clipper Burn	23 Jun-5 Jul	M	25	B
	83F339	Erythema	22-26 Jun	V	10	B
		Clipper Burn	1-4 Jul	SL	5	RHL
	83F342	Erythema	22 Jun	V	5	B
		Scaling	26 Jun-4 Jul	V	5	B,O
		Clipper Burn	23,24 Jun	V	5	RHL
	83F347	Clipper Burn	2-5 Jul	V	5	B
	83F356	Erythema	22,23 Jun	M	25	B
		Scaling	27,28 Jun	V	5	O
		Clipper Burn	1-4 Jul	V	5	B

Severity	Exposed Area	Location	
V = Very Slight	5 = < 5%	A = Abdomen	O = Abrasions
SL = Slight	10 = < 10%	B = Back	T = Teat
M = Moderate	25 = < 25%	C = Thorax	U = Umbilicus
D = Defined	49 = < 50%	F = Flank	RHL = Right Hind Leg
S = Severe	51 = > 50%	S = Lateral (Side)	

TABLE 2B

DERMAL TOXICITY POTENTIAL OF HOLSTON COMPOUNDS IN RABBITS

SUMMARY OF ACUTE DERMAL TOXICITY SIGNS

GLP Study #82038 — Group 2 Virgin DMSO

	Animal Number	Signs of Dermal Irritation	Dates (1983)	Severity (max)	Exposed Area (max)	Location
MALES (N=5)	83F113	None Observed	N/A	N/A	N/A	N/A
	83F114	Scaling	25-27 Mar	V	5	0
	33F115	Scaling	25-27 Mar	V	5	0
	83F129	None Observed	N/A	N/A	N/A	N/A
	83F131	None Observed	N/A	N/A	N/A	N/A
FEMALES (N=5)	83F337	Erythema	22 Jun	SL	5	0
		Scaling	24,25,27-30 Jul	V	5	0
			1-5 Jul	V	5	0
		Clipper Burn	28,29 Jun	M	10	B
	83F343	Erythema	24-29 Jun	SL	10	B
		Scaling	28,29 Jun	V	5	0
		Clipper Burn	23-25 Jun,	V	5	LS
			30 Jun, 1-4 Jul	V	5	B
	83F349	Scaling	26,27 Jun	SL	5	LS
		Clipper Burn	24,25 Jun,	SL	10	RHL,B
			27-30 Jun, 1-5 Jul	D	10	B
	83F353	Scratch	23-25 Jun	SL	5	RS
		Scaling	26,27 Jun	V	5	RS,O
		Clipper Burn	27-30 Jun, 1-4 Jul	M	10	B
	83F354	Clipper Burn	1-4 Jul	SL	5	LS,B

Severity	Exposed Area	Location	
V = Very Slight	5 = < 5%	A = Abdomen	O = Abrasions
SL = Slight	10 = < 10%	B = Back	T = Teat
M = Moderate	25 = < 25%	C = Thorax	U = Umbilicus
D = Defined	49 = < 50%	F = Flank	RHL = Right Hind Leg
S = Severe	51 = > 50%	S = Lateral (Side)	

TABLE 2C

DERMAL TOXICITY POTENTIAL OF HOLSTON COMPOUNDS IN RABBITS

SUMMARY OF ACUTE DERMAL TOXICITY SIGNS

GLP Study #82038 — Group 3 DMSO Evaporator Sludge

	Animal Number	Signs of Dermal Irritation	Dates (1983)	Severity (max)	Exposed Area (max)	Location
MALES (N=5)	83F123	Erythema	22,23 Mar	SL	5	B
	83F125	None Observed	N/A	N/A	N/A	N/A
	83F126	None Observed	N/A	N/A	N/A	N/A
	83F127	None Observed	N/A	N/A	N/A	N/A
	83F132	None Observed	N/A	N/A	N/A	N/A
FEMALES (N=5)	83F336	Erythema	22 Jun	SL	5	0
		Scaling	25-30 Jun	V	10	B,0
	83F341	Erythema	29 Jun	V	5	B
		Scaling	29,30 Jun, 2-4 Jul	V	5	B,0
	83F345	Scaling	24-30 Jun, 1-5 Jul	V	5	B,0
	83F348	Scaling	25-29 Jun	V	5	0
		Clipper Burn	22-24 Jun, 2-5 Jul	M	5	RHL,B
		Scar	5 Jul	V	5	B
	83F351	Scaling	24-26 Jun	V	5	0

Severity

V = Very Slight
SL = Slight
M = Moderate
D = Defined
S = Severe

Exposed Area

5 = < 5%
10 = < 10%
25 = < 25%
49 = < 50%
51 = > 50%

Location

A = Abdomen
B = Back
C = Thorax
F = Flank
S = Lateral (Side)
0 = Abrasions
T = Teat
U = Umbilicus
RHL = Right Hind Leg

TABLE 2D

DERMAL TOXICITY POTENTIAL OF HOLSTON COMPOUNDS IN RABBITS

SUMMARY OF ACUTE DERMAL TOXICITY SIGNS

GLP Study #82038 — Group 4 Saline Control

	Animal Number	Signs of Dermal Irritation	Dates (1983)	Severity (max)	Exposed Area (max)	Location
MALES (N=5)	83F118	None Observed	N/A	N/A	N/A	N/A
	83F121	None Observed	N/A	N/A	N/A	N/A
	83F122	None Observed	N/A	N/A	N/A	N/A
	83F130	None Observed	N/A	N/A	N/A	N/A
	83F133	None Observed	N/A	N/A	N/A	N/A
FEMALES (N=5)	83F338	Erythema	22 Jun	V	5	0
		Scaling	24-27 Jun, 29 Jun	V	5	0
		Scabbing	5 Jul	SL	5	B
		Clipper Burn	28-30 Jun, 1-4 Jul	D	10	B
	83F340	Scaling	24,27-30 Jun, 2-4 Jul	SL	5	B,0
	83F346	Erythema	22,24,25 Jun	SL	5	B
		Edema	24 Jun	V	5	B
		Clipper Burn	24-28 Jun, 2-5 Jul	SL	5	B,N
	83F352	Erythema	24-26 Jun	V	10	B
		Clipper Burn	1-5 Jul	M	5	N
	83F355	Erythema	25,26 Jun	SL	5	B
		Clipper Burn	1-5 Jul	SL	10	B

Severity	Exposed Area	Location	
V = Very Slight	5 = < 5%	A = Abdomen	0 = Abrasions
SL = Slight	10 = < 10%	B = Back	T = Teat
M = Moderate	25 = < 25%	C = Thorax	U = Umbilicus
D = Defined	49 = < 50%	F = Flank	RHL = Right Hind Leg
S = Severe	51 = > 50%	S = Lateral (Side)	

Treatment of Animal Disease and Injury

Rabbits were placed on theraputic levels of sulfaquinoline (3.2 ml per 236 ml bottle) of drinking water for coccidiosis prophylaxis during quarantine. They did not receive sulfaquinoline after they were placed in the GLP suite.

Gross Pathological Observations

It does not appear that the application of DMSO Recycle Solvent, Virgin DMSO, or DMSO Evaporator Sludge to close-clipped abraded skin of male and female rabbits for 24 hours caused or intensified the inflammatory response that could be detected 14 days after application. A report of gross pathological observations appears in Appendix E.

DISCUSSION

The acute dermal toxicity test evaluates the potential for systemic toxic effects of a given substance. There were no deaths for rabbits dosed at 2 ml/kg body weight during the acute dermal toxicity test. The acute dermal toxicity test also revealed that the Holston Compounds (DMSO Recycle Solvent, Virgin DMSO, and DMSO Evaporator Sludge) did not cause clinical signs of systemic toxicity when applied in 2 ml/kg quantities to approximately 10% of the rabbits' body surface. The Holston Compounds did produce a slight dermal irritation; however, the saline control group animals, exhibited a similar dermal response. This lack of a differential dermal response versus the control group, suggests that the Holston Compounds possess minimal potential for acute dermal toxicity.

CONCLUSION

The Holston Compounds caused no clinical signs and only a slight dermal irritation to the clipped skin when rabbits were subjected to a 24-hour period of topical exposure and observed for 14 days. However, a similar response was observed in the control group. Therefore, it can be concluded that the Holston Compounds produce minimal dermal toxicity under the conditions of this study.

RECOMMENDATION

The Holston Compounds should undergo additional dermal irritation and sensitization testing because of the extreme tissue penetrative properties of their major component, DMSO.

REFERENCES

1. McNamara BP, Averill HP, Owens EJ, Callahan JF, Fairchild DG, Cinchta HP, Rengstroff RH, Biskup RK. The toxicology of cyclotrimethylenetrinitramine (RDX) and cyclotetremethylenetetranitramine (HMX) solutions in dimethyl sulfoxide [DMSO], cyclohexenone, and acetone. Edgewood Arsenal Technical Report, EB-TR-73040, April 1974.

2. Cholakis JM, Wong LC, Van Goethern DL, Minor J, Short R, Spring H, Ellis, HV III. Mammalian toxicological evaluation of RDX. Technical Report, U.S. Army Medical Research and Development Command, Fort Detrick, MD, September 1980.

3. Stidham, BR. Analysis of waste waters for organic compounds unique to RDX/HMX manufacturing processing. U.S. Army Medical Research and Development Command, Washington, DC, Technical Report, December 1979.

4. Tyson CA, Dilley JV, Sasmore DP, Spanggord RJ, Newell GW, Ducre JC. Single-dose and repeated-exposure toxicity of a complex waste water from munitions manufacturing plants. J Toxicol Environ Health, 1982; 9:545-564. 1982.

5. Environmental Protection Agency. Good Laboratory Practices proposed regulations (40 CFR 770,771,772) and preamble as published in the Federal Register, 22 Aug 78, 9 May 79, 26 Jul and 18 Apr 80, (45 FR26373).

6. Food and Drug Administration. Good Laboratory Practices regulations (21 CFR58) and preamble, published in the Federal Register, 22 Dec 78 (45 FR59986-60025).

7. Interagency Regulatory Liaison Group Testing Standards and Guidelines Work Group. Recommended guideline for acute dermal toxicity test. 1981.

8. Association of Food and Drug Officials of U.S. Appraisal of the safety of chemicals in foods, drugs and cosmetics (4th printing). 1959.

9. Committee for the Revision of NAS Publication 1138. Committee on Toxicology, National Research Council. Principles and procedures for evaluating the toxicology of household substances. Prepared for the Consumer Product Safety Commission. Washington, DC: National Academy of Sciences, 1977.

10. Bedford CD, Deas BD, Broussard MM, Geigl MA, Marynowski CW. Preparation and purification of multigram quantities of TAX and SEX. US Army Medical Research and Development Command, Fort Detrick, MD, Third Phase, Final Report, December 1981.

11. Haley TJ, Hunziker J. Instrument for producing standardized skin abrasion. J Pharm Sci 1974;63:106.

LIST OF APPENDICES

Page

Appendix A, Chemical Analysis Data.....17

Appendix B, Animal Data.....29

Appendix C, Historical Listing of Study Events.....33

Appendix D, Dermal Irritation Scores.....35

Appendix E, Pathology Report.....41

Toxicity Test Sample Composition[a]

Concentration by HPLC, g/l

Sample	RDX[b]	HMX[c]	TAX[d]	SEX[e]	%H_2O	%DMSO
Virgin DMSO[f]	0	0	0	0	0.63[g]	99.37
DMSO Recycle Solvent[h]	24.188	39.542	0.263	0	35.48[i]	58.64[j]
DMSO Evaporator Sludge[f]	0.548	0.942	3.521	0	5.35[i]	94.19[j]

Calculated Data In Weight Percent[a]

Sample	RDX	HMX	TAX	SEX	H_2O	DMSO
Virgin DMSO	0	0	0	0	0.63	99.37
DMSO Recycle Solvent	2.22	3.64	0.02	0	35.48	58.64
DMSO Evaporator Sludge	0.05	0.09	0.32	0	5.35	94.19

[a] Data supplied by sponsor

[b] RDX: Hexahydro-1,3,5-Trinitro-1,3,5-Triazine

[c] HMX: Octhydro-1,3,5,7-Tetranitro-1,3,5,7-Tetrazocine

[d] TAX: 1-Acetylhexahydro-3,5-Dinitro-1,3,5-Triazine

[e] SEX: 1-Acetyloctahydro-3,5,7-Trinitro-1,3,5,7-Tetrazocine

[f] At ambient temperature.

[g] By Karl Fisher

[h] Analysis of equilibrium liquid at 40 C.

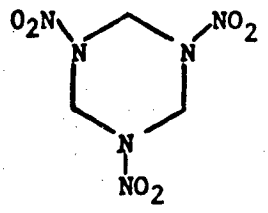
[i] Water content calculated by difference.

[j] DMSO content by gas chromatography using Virgin DMSO sample as the standard.

1. Chemical name: Hexahydro-1,3,5-Trinitro-1,3,5-Triazine, Cyclotrimethylenetrinitramine, Cyclonite Hexogen, RDX

   Chemical Abstract Service Registry Number: 121-82-4

   Structural formula:



   Empirical formula: $C_3H_6N_6O_6$

   Molecular weight: 222.13 g/mole

   Physical State: White crystals varying in size

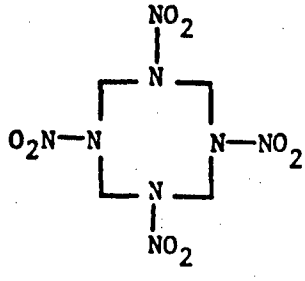
   Melting point: 200-203 C

   Manufacturer: Holston Army Ammunition Plant
   Kingsport, TN

2. Chemical name: Octahydro-1,3,5,7-Tetranitro-1,3,5,7-Tetrazine HMX, Cyclotetramethylenetrinitramine

   Chemical Abstract Service Registry Number: 2691-41-0

   Structural formula:



Empirical formula: $C_4H_8O_8N_8$

Molecular weight: 296.17 g/mole

Physical state: White crystals of varying size

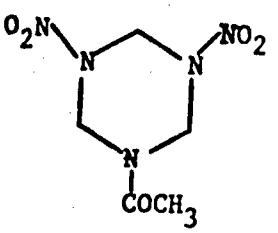
Melting point: 280 C

Manufacturer: Holston Army Ammunition Plant
Kingsport, TN

3. Chemical name: Hexahydro-1-(N)-Acetyl-3,5-Dinitro-1,3,5-Triazine, TAX

Chemical Abstract Service Registry Number: 14168-42-4

Structural formula:



Empirical formula: $C_5H_9O_5N_5$

Molecular weight: 219.17 g/mole

Physical state: White crystals of varying size

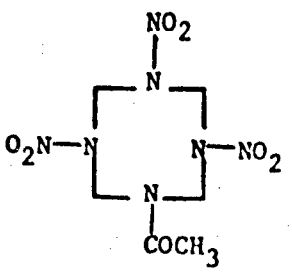
Melting point: 156 C

Manufacturer: By-product of the production/processing of HMX/RDX at the Holston Army Ammunition Plant, Kingsport, TN

4. Chemical name: Octahydro-1-(N)-Acetyl-3,5,7-Trinitro-1,3,5,7-Tetrazine, SEX

Chemical Abstract Service Registry Number: 13980-00-2

Structural formula:



Empirical formula: $C_6H_{11}O_7N_7$

Molecular weight: 293.21 g/mole

Physical State: White crystals of varying size

Melting point: 224.2-224.7 C

Manufacturer: By-product of the production/processing of HMX/RDX at the Army Ammunition Plant, Kingsport, TN

5. Chemical name: Dimethyl Sulfoxide (DMSO)

Chemical Abstract Service Registry Number : 00006-76-85

Structural formula: C_2H_6SO

Empirical structure: $CH_3-\overset{\overset{O}{\|}}{S}-CH_3$

Molecular weight: 78.02 g/mole

Physical state/color: Clear transparent liquid.

Freezing point: 18.55 C

Boiling point: 189 C

Contaminants: Water 0.63 percent

Manufacturer: Crown Zellerbach Corporation
Chemical Products Division
Camas, WA 98607

6. Chemical name: Dimethy Sulfoxide (DMSO) reagent grade

Chemical Abstract Service Registry Number: 00006-76-85

Structural formula: $CH_3-\overset{\overset{O}{\|}}{S}-CH_3$

Empirical formula: C_2H_6SO

Physical state: Clear transparent liquid

Freezing point: 18.3 C

Boiling point: 189 C

Density: 1.095 g/ml

Contaminants: Water 0.08%

Manufacturer: J.T. Baker Chemical Co.
Phillipsburg, NJ 08805

HOLSTON DEFENSE CORPORATION

WEST STONE DRIVE

KINGSPORT, TENNESSEE 37660

TELEPHONE: AREA CODE 615 247-9111

June 22, 1983

Contracting Officer's Representative
Holston Army Ammunition Plant
Kingsport, Tennessee 37660

Dear Sir:

Subject: DMSO Process Stream Toxicological Testing

Reference: USAMBRDL Letter to Commander, HSAAP, "DMSO Munition Process Solvent Toxicology Studies Laboratory Monitoring Visits and Technical Status Review Meetings," dated November 23, 1982

1. The meetings referred to in the above reference were attended as requested. At that time the toxicity studies at both LAIR and LEHR were just getting under way, and the meetings were used to review preliminary results then available as well as plans for completing the studies. Holston was also involved in a characterization screening study of the same test samples in an attempt to identify potentially toxic compounds which might be present and could contribute to the toxic or mutagenic results observed.

   The test samples had been previously analyzed for composition at Holston and shipped to LAIR. At the referenced meeting, Col. Fruin requested that in addition Holston furnish both the results of the characterization screening study and the details of the analytical methods used to perform the original quantitative analyses on the test samples at Holston. The screening study at Holston has now been completed, and the requested information is hereby transmitted.

2. The characterization screening study was performed on the composite recycle solvent sample from the DMSO pilot plant. Also, production crude/water-washed RDX and HMX samples were subjected to analyses to determine if any unusual compounds could be detected for comparison with any found in the DMSO sample. HPLC methods were used during the screening procedure varying the columns, solvent systems, wavelengths, and the other parameters such that any contaminant peaks found could be identified by component retention time.

   Initial HPLC analysis of the recycle solvent sample showed very large concentrations of RDX and HMX which interfered with analysis of other components. The sample was treated to remove the bulk of the RDX and HMX by heating to 40°C and then quenching one to one with water. The decanted liquid was then subjected to the remainder of the screening

study analyses. The sample was examined by several HPLC systems available at Holston which are normally used to analyze RDX, HMX, and related nitramines found in various plant process streams and products. These are presented in Attachments II and III. Other HPLC conditions presented in Attachment I, which do not represent proven HPLC methods, were also used to get as much system variability as possible. Note that Holston does not guarantee these results since these procedures in Attachment I were used only for screening and qualitative purposes. It should also be realized that most of Holston's routine procedures are used to detect nitramine or related compounds. Other impurities may not have been detected by these methods. The only compounds detected using any of the systems were RDX, HMX, SEX, and TAX. HPLC retention times for these compounds matched the known retention times for RDX, HMX, SEX, and TAX. Attachment I also presents the results obtained. Analysis of crude RDX and HMX by the methods described in Attachment II yielded no evidence of the presence of compounds other than RDX, HMX, and SEX.

3. Quantitative analyses of the test samples were performed by HPLC. Since no reliable method for direct analysis of DMSO by either HPLC or GC has been developed, DMSO values are by difference. Attachment III presents an outline of the quantitative methods used.

4. This information should be transmitted to the following:

Col. John Fruin
Building 1110
Presidio of San Francisco
California 94129

Capt. James Carroll
USAMBRDL
Building 568
Fort Detrick
Frederick, Maryland 21701

Raymond Goldstein
ARRADCOM
Picatinny Arsenal
Dover, New Jersey

Yours very truly,

HOLSTON DEFENSE CORPORATION

M B Knowles

M B Knowles
Plant Manager

Attachments (3)

ATTACHMENT I

Mullen--30 HPLC Analysis of RDX & HMX Recycled DMSO

HPLC Parameters	Components Detected
1. Column: Waters CN, 1/4" x 12" ss Detector: UV at 254 NM Solvent System: 70% iso-octane 15% chloroform 10% acetonitrile 5% methanol Flow Rate: 3.0 ml/min Injection Volume: 10 microliters	RDX HMX SEX
2. Column: LiChrosorb-Amine, 1/4" x 12" ss Detector: UV, 230-260 nm in 10 nm increments Solvent System: 70% iso-octane 15% chloroform 10% acetonitrile 5% methanol Flow Rate: 3.0 ml/min Injection Volume: 10 microliters	RDX HMX
3. Column: LiChrosorb-Diol, 1/4" x 12" ss Detector: UV, 230-260 nm in 10 nm increments Solvent System: 70% iso-octane 15% chloroform 10% acetonitrile 5% methanol Flow Rate: 3.0 ml/min Injection Volume: 10 microliters	RDX HMX
4. Column: Waters CN, 1/4" x 12" ss Detector: UV at 254 nm Solvent System: 70% water 30% methanol Flow Rate: 2.5 ml/min Injection Volume: 10 microliters	RDX HMX TAX
5. Column: Waters CN, 1/4" x 12" ss Detector: UV, 215-290 nm in 10 nm increments Solvent System: 80% water 20% methanol Flow Rate: 2.5 ml/min Injection Volume: 10 microliters	RDX HMX TAX
6. Column: Waters CN, 1/4" x 12" ss Detector: UV, 215-290 nm in 10 nm increments Solvent System: 60% water 40% methanol Flow Rate: 2.5 ml/min Injection Volume: 10 microliters	RDX HMX TAX

	HPLC Parameters	Components Detected
7.	Column: Waters CN, 1/4" x 12" ss Detector: UV at 254 nm Solvent System: 50% water 50% methanol Flow Rate: 2.5 ml/min Injection Volume: 10 microliters	No component separation
8.	Column: LiChrosorb-Diol, 1/4" x 12" ss Detector: UV at 254 nm Solvent System: 80% water 20% methanol Flow Rate: 2.5 ml/min Injection Volume: 10 microliters	No component separation
9.	Column: LiChrosorb-Amine, 1/4" x 12" ss Detector: UV at 254 nm Solvent System: 80% water 20% methanol Flow Rate: 2.5 ml/min Injection Volume: 10 microliters	No component separation
10.	Column: LiChrosorb-RP18, 1/4" x 12" ss Detector: UV, 215-290 nm in 10 nm increments Solvent System: 80% water 20% methanol Flow Rate: 2.5 ml/min Injection Volume: 10 microliters	RDX HMX TAX SEX
11.	Column: LiChrosorb-RP18 1/4" x 12" ss Detector: UV at 254 nm Solvent System: 60% water 40% methanol Flow Rate: 2.5 ml/min Injection Volume: 10 microliters	No component separation
12.	Column: LiChrosorb-RP 8 1/4" x 6" ss Detector: UV, 215-290 nm in 10 nm increments Solvent System: 80% water 20% methanol Flow Rate: 2.0 ml/min Injection Volume: 10 microliters	RDX HMX TAX SEX
13.	Column: LiChrosorb-RP 8 1/4" x 6" ss Detector: UV at 254 nm Solvent System: 60% water 40% methanol Flow Rate: 2.0 ml/min Injection Volume: 10 microliters	No component separation

ATTACHMENT II

HPLC Analysis of Crude RDX

HPLC Parameters	Components Detected
Column: Waters CN, 1/4" x 12" ss	
Detector: UV, 215-290 nm in 10 nm increments	RDX HMX SEX
Solvent System: 70% iso-octane 15% chloroform 10% acetonitrile 5% methanol	
Flow Rate: 3.0 ml/min	
Injection Volume: 10 microliters	

HPLC Analysis of Crude HMX

HPLC Parameters	Components Detected
Column: Waters CN, 1/4" x 12" ss	
Detector: UV, 215-290 nm in 10 nm increments	RDX HMX SEX
Solvent System: 70% iso-octane 15% chloroform 10% acetonitrile 5% methanol	
Flow Rate: 3.0 ml/min	
Injection Volume: 10 microliters	

ATTACHMENT III

Quantitative Analysis of DMSO/Explosives Samples

Sample Preparation

1. Weigh representative liquid sample.
2. Evaporate sample to dryness - weigh dried sample.
3. Add acetonitrile to sample sufficient to completely dissolve all solids.
4. Analyze for RDX, HMX, and SEX using Procedure A below.
5. Analyze for TAX using Procedure B below.

Procedure A - HPLC

Column: Waters CN, 1/4" x 12" ss (Waters No. 84082)
Detector: UV at 254 nm
Solvent System: 70% iso-octane
15% chloroform
10% acetonitrile
5% methanol
Flow Rate: 3.0 ml/min
Injection Volume: 10 microliters
Typical Retention Times (seconds): RDX - 195
SEX - 365
HMX - 423

Procedure B - HPLC

Column: Waters CN, 1/4" x 12" ss (Waters No. 84082)
Detector: UV at 254 nm
Solvent System: 80% water
20% methanol
Flow Rate: 2.5 ml/min
Injection Volume: 10 microliters

DMSO/Water Content

Karl Fischer titration was used to determine the water content of the liquid recycle solvent. DMSO was determined by difference as below:

$$\% \text{ DMSO} = 100\% - \% \text{ Solids} - \% \text{ Water}$$

ANIMAL DATA

MALES

Species: Rabbit

Strain: New Zealand White

Rationale for selection: The New Zealand White Rabbit is a proven mammalian model for acute dermal studies because of its size, ease of handling, restraint, and skin permeability.

Source: Elkhorn Rabbitry
565 Starr Way
Watsonville, CA 95076

Pretest Conditioning:

a. Arrival at LAIR 17 Feb 83, quarantine time 14 days.

b. Animals reclipped before dosing.

c. Animals given sulfaquinoline (SQ) during quarantine, at a standard dosage of 3.2 ml SQ per 236 ml water bottle ad lib for seven days.

Restraint: Manual restraint during application. Animals left their bandages alone over the 24-hour period.

Sex: Male

Age: Young adult

Method of Randomization: Manually by Random Numbers Table

Animals in Each Group: 5 males per test chemical; 5 males in wrapped saline control.

Condition of Animals at Start of Study: Normal

Mean Weight (± 1 standard deviation) at Dosing:

2463 (± 86) g for TP013 group
2509 (± 116) g for TP014 group
2598 (± 199) g for TP015 group
2408 (± 154) g for control group

Mean Weight (± 1 standard deviation) at Sacrifice:

2446 (± 150) g for TP013 group
2421 (± 146) g for TP014 group
2633 (± 130) g for TP015 group
2479 (± 77) g for control group

Identification Procedures: Ear tattooed IAW SOP OP-ARG-1

FEMALES

Species: Rabbit

Strain: New Zealand White

Rationale for selection: The New Zealand White Rabbit is a proven mammalian model for acute dermal studies because of its size, ease of handling, restraint, and skin permeability.

Source: Elkhorn Rabbitry
565 Starr Way
Watsonville, CA 95076

Pretest Conditioning:

a. Arrival at LAIR 2 Jun 83, quarantine time 14 days.

b. Animals clipped the day before dosing.

c. Animals given sulfaquinoline (SQ) during quarantine, at a standard dosage of 3.2 ml SQ per 256 ml water bottle ad lib for seven days.

Restraint: Manual restraint during application. Animals left their bandages alone over the 24-hour period.

Sex: Female

Age: Young adult

Method of Randomization: Manually by Random Numbers Table

Animals in Each Group: 5 females per test chemical; 5 females in wrapped saline control.

Condition of Animals at Start of Study: Normal

Mean Weight (± 1 standard deviation) at Dosing:

2971 (± 257) g for TP013 group
2964 (± 363) g for TP014 group
2854 (± 243) g for TP015 group
3081 (± 266) g for control group

Mean Weight (± 1 standard deviation) at Sacrifice:

2861 (± 178) g for TP013 group
2930 (± 340) g for TP014 group
2895 (± 198) g for TP015 group
3039 (± 220) g for control group

Identification Procedures: Several females arrived previously tattooed. They were cross referenced IAW SOP-OP-ARG-1. The remaining females were tattooed IAW SOP-OP-ARG-1.

HISTORICAL LISTING OF STUDY EVENTS

MALES

Date	Event
17 Feb 83	Male rabbits arrived at LAIR. They were checked for illness and quarantined in Room R31409.
7 Mar 83	21 males were removed from quarantine, separated into test groups and prepared for study.
21 Mar 83	Rabbits were dosed according to SOP-OP-STX-30. The clipped areas were abraded and test substance applied. Rabbits were observed frequently after dosing. Clinical signs were recorded twice after dosing.
22 Mar 83	Bandaging materials were removed. Animals were observed.
22 Mar 83- 4 Apr 83	Clinical observations were recorded once a day.
4 Apr 83	Animals were not fed; they were observed and weighed. Euthanasia and necropsies were performed. Several cutaneous sites were selected for histopathological observation.

HISTORICAL LISTING OF STUDY EVENTS

FEMALES

Date	Event
2 Jun 83	Female rabbits arrived at LAIR. They were checked for illness and quarantined in Room RS1409.
16 Jun 83	21 females were removed from quarantine, separated into test groups and prepared for study.
17,20 Jun 83	Hair was clipped from the back.
21 Jun 83	Rabbits were dosed according to SOP-OP-STX-30. The clipped areas were abraded and test substance applied. Rabbits were observed frequently after dosing. Clinical signs were recorded twice after dosing.
22 Jun 83	Bandaging materials were removed. Animals were observed.
22 Jun 83-5 Jul 83	Clinical observations were recorded once a day.
5 Jul 83	Animals were not fed; they were observed and weighed, then euthansia and necropsies were performed. Several cutaneous sites selected for histopathological observation.

LIST OF TABLES

Page

Table 1, Acute Dermal Toxicity Irritation Scores
TP013, TP014 (Males).....37

Table 2, Acute Dermal Toxicity Irritation Scores
TP015, Saline Control (Males).....38

Table 3, Acute Dermal Toxicity Irritation Scores
TP013, TP014 (Females).....39

Table 4, Acute Dermal Toxicity Irritation Scores
TP015, Saline Control (Females).....40

APPENDIX D

GLP Study No. 82038

Dose Date: 21 March 1983 Test Substance: TPO13, TPO14

TABLE 1
ACUTE DERMAL TOXICITY
IRRITATION SCORES FOR SKIN (24h, 48h, 72h)

	Erythema			Edema		
TPO13 Male No.	24h	48h	72h	24h	48h	72h
83F119	0	0	0	0	0	0
83F120	0	0	0	0	0	0
83F124	0	0	0	0	0	0
83F128	0	0	0	0	0	0
83F134	0	0	0	0	0	0
TPO14 Male No.						
83F113	0	0	0	0	0	0
83F114	0	0	0	0	0	0
83F115	0	0	0	0	0	0
83F129	0	0	0	0	0	0
83F131	0	0	0	0	0	0

Erythema Formation:

	Value
None	0
Very slight	1
Well-defined	2
Moderate	3
Severe	4

Edema Formation:

	Value
None	0
Very slight	1
Slight	2
Moderate	3
Severe	4

Initial/Date: 22, 23, 24 Mar 83 Lawrence Mullen

GLP Study No. 82038

Dose Date: 21 March 1983 Test Substance: TPO15, Saline Control

TABLE 2
ACUTE DERMAL TOXICITY
IRRITATION SCORES FOR SKIN (24h, 48h, 72h)

	Erythema			Edema		
TPO15 Male No.	24h	48h	72h	24h	48h	72h
83F123	2	1	0	0	0	0
83F125	0	0	0	0	0	0
83F126	0	0	0	0	0	0
83F127	0	0	0	0	0	0
83F132	0	0	0	0	0	0
Saline Control Male No.						
83F118	0	0	0	0	0	0
83F121	0	0	0	0	0	0
83F122	0	0	0	0	0	0
83F130	0	0	0	0	0	0
83F133	0	0	0	0	0	0

Erythema Formation:

	Value
None	0
Very slight	1
Well-defined	2
Moderate	3
Severe	4

Edema Formation:

	Value
None	0
Very slight	1
Slight	2
Moderate	3
Severe	4

Initial/Date: 22, 23, 24 March 83 Lawrence Mullen

GLP Study No. 82038

Dose Date: 21 June 1983 Test Substance: TP013, TP014

TABLE 3
ACUTE DERMAL TOXICITY
IRRITATION SCORES FOR SKIN (24h, 48h, 72h)

	Erythema			Edema		
TP013 Female No.	24h	48h	72h	24h	48h	72h
83F335	0	0	0	0	0	0
83F339	1	1	1	0	0	0
83F342	1	0	0	0	0	0
83F347	0	0	0	0	0	0
83F356	2	1	0	0	0	0
TP014 Female No.						
83F337	1	0	0	0	0	0
83F343	0	0	1	0	0	0
83F349	0	0	0	0	0	0
83F353	0	0	0	0	0	0
83F354	0	0	0	0	0	0

Erythema Formation:

	Value
None	0
Very slight	1
Well-defined	2
Moderate	3
Severe	4

Edema Formation:

	Value
None	0
Very slight	1
Slight	2
Moderate	3
Severe	4

Initial/Date: 22, 23, 24 Jun 83 Lawrence Mullen

GLP Study No. 82038

Dose Date: 21 June 1983 Test Substance: TP015, Saline Control

TABLE 4
ACUTE DERMAL TOXICITY
IRRITATION SCORES FOR SKIN (24h, 48h, 72h)

	Erythema			Edema		
TP015 Female No.	24h	48h	72h	24h	48h	72h
83F336	1	0	0	0	0	0
83F341	0	0	0	0	0	0
83F345	0	0	0	0	0	0
83F348	0	0	0	0	0	0
83F351	0	0	0	0	0	0
Saline Control Female No.						
83F338	1	0	0	0	0	0
83F340	0	0	0	0	0	0
83F346	1	0	1	0	0	1
83F352	0	0	1	0	0	0
83F355	0	0	0	0	0	0

Erythema Formation:

	Value
None	0
Very slight	1
Well-defined	2
Moderate	3
Severe	4

Edema Formation:

	Value
None	0
Very slight	1
Slight	2
Moderate	3
Severe	4

Initial/Date: 22, 23, 24 Jun 83 Lawrence Mullen

PATHOLOGY REPORT

GLP Study 82038

Acute Dermal Toxicity (Limit Test) of DMSO Recycle Solvent (TP013), Virgin DMSO (TP014), and DMSO Evaporator Sludge (TP015) in Male and Female New Zealand White Rabbits

History: The purpose of this study was to determine the acute dermal toxicity of TP013, TP014, and TP015 in male and female New Zealand White Rabbits. Two ml/kg of tested material was applied to the clipped and abraded skin of the rabbits in groups 1 - 3 for 24 hours. The skin of the saline control rabbits in group 4 was clipped and abraded.

After a 14-day observation period for males and a 14-day observation period for females, the rabbits were submitted for necropsy. They were killed by exsanguination from severed axillary vessels while under anesthesia produced by intravenous injection of pentobarbitol. Complete gross necropsies were performed and two specimens of skin from each exposed area were fixed in neutral bufferd formalin, embedded in paraffin, sectioned at approximately 6 micrometers, and stained with hematoxylin and eosin for microscopic examination.

Gross necropsy findings: No gross lesions were observed in any of the controls or rabbits exposed to the tested compounds.

Microscopic findings: Three types of microscopic lesions were observed in the rabbit skin from the clipped and abraded sites. The most common type lesion was a minimal, mild, or mild-moderate, focal, multifocal, focally extensive, or diffuse infiltration of macrophages, lymphocytes and plasma cells (collectively referred to as mononuclear inflammatory cells) in the upper half of the dermis immediately beneath the epidermis. On occasion, some infiltrates contained a prominent heterophil component. The second most common lesion was minimal, mild, or moderate, focal, multifocal, or diffuse epidermal hyperplasia. The epidermis in these foci was 2 to 3 times the thickness of the more normal epidermis. The third lesion was mild, focal, or multifocal purulent exudate within the keratin covering the epidermis. Microscopic findings in each skin section examined are tabulated in Table I or II. Table III is a summary of the incidence of skin lesions by sex and experimental group.

Inflammatory cell infiltration was present in the upper dermis of 3/5* male and 4/5 female rabbits exposed to TP013, 2/5 male and 4/5 female rabbits exposed to TP014, 4/5 male and 4/5 female rabbits exposed to TP015, and 2/5 male and 4/5 female saline controls. A focus of

*Number of rabbits affected/Number of rabbits in treatment group

mononuclear inflammatory cells in the dermis of 1 of the female rabbits exposed to TP014 surrounded a bare hair shaft. Epidermal hyperplasia was present in 2/5 female rabbits exposed to TP013 and 1/5 male rabbits exposed to TP014. Purulent exudate was present in the keratin covering the epidermis in 2/5 female rabbits exposed to TP013. The skin was essentially normal in 2/5 male and 1/5 female rabbits exposed to TP013, 3/5 male and 1/5 female rabbits exposed to TP014, 1/5 male and 1/5 female rabbits exposed to TP015, and 3/5 male and 1/5 female saline controls. The type, pattern, and severity of lesions in the male and female rabbits on this study indicate that they are background findings and are most likely not due to or aggrevated by the tested materials. The relatively increased severity of the inflammatory cell infiltrate and the epidermal hyperplasia and the purulent surface exudate in the female rabbits exposed to TP013 is suggestive of but not definitive of an irritant effect of the TP013.

In summary, there is no clear indication that the application of TP013, TP014, or TP015 to close clipped abraded skin of male or female rabbits for 24 hours causes or intensifies an inflammatory response in the skin that can be detected 14 days after application.

Glen E. Marrs, Jr.

GLEN E. MARRS, JR., DVM, MS
Diplomate, A.C.V.P.
MAJ, VC
Assistant Chief, Pathology Services Group
Division of Research Support

12 September 1983

GLP Study 82-038

Table I

Acute Dermal Toxicity (Limit Test) of DMSO Recycle Solvent (TP013), Virgin DMSO (TP014) and DMSO Evaporator Sludge (TP015) in Male New Zealand White Rabbits

Group 1 - 2 ml/kg TP013

83F00119	33691-1	Essentially normal skin.
	33691-2	Mononuclear inflammatory cell infiltrate, focal, minimal
83F00120	33692-1	Essentially normal skin
	33692-2	Essentially normal skin
83F00124	33696-1	Mononuclear inflammatory cell infiltrate, focal, minimal.
	33696-2	Essentially normal skin
83F00128	33700-1	Essentially normal skin
	33700-2	Essentially normal skin
83F00134	33706-1	Essentially normal skin
	33706-2	Mononuclear inflammatory cell infiltrate, focal, minimal

Group 2 - 2 ml/kg TP014

83F00113	33687-1	Essentially normal skin
	33687-2	Essentially normal skin
83F00114	33688-1	Mononuclear inflammatory cell infiltrate, focal, mild Epidermal hyperplasia, focal, minimal
	33688-2	Essentially normal skin
83F00115	33689-1	Essentially normal skin
	33689-2	Essentially normal skin
83F00129	33701-1	Mononuclear and heterophilic inflammatory cell infiltrate, focal, minimal
	33701-2	Mononuclear and heterophilic inflammatory cell infiltrate, focal, minimal
83F00131	33703-1	Essentially normal skin
	33703-2	Essentially normal skin

GLP Study 82-038, Males (cont'd)

Group 3 - 2 ml/kg TP015

83F00123	33695-1	Essentially normal skin
	33695-2	Essentially normal skin
83F00125	33697-1	Mononuclear inflammatory cell infiltrate, focal, minimal
	33697-2	Mononuclear inflammatory cell infiltrate, focal, minimal
83F00126	33698-1	Mononuclear inflammatory cell infiltrate, focal, minimal
	33698-2	Essentially normal skin
83F00127	33699-1	Mononuclear inflammatory cell infiltrate, focal, minimal
	33699-2	Essentially normal skin
83F00132	33704-1	Mononuclear inflammatory cell infiltrate, focal, minimal
	33704-2	Essentially normal skin

Group 4 - Saline Control

83F00118	33690-1	Essentially normal skin
	33690-2	Mononuclear inflammatory cell infiltrate, diffuse, minimal
83F00121	33693-1	Essentially normal skin
	33693-2	Essentially normal skin
83F00122	33694-1	Mononuclear inflammatory cell infiltrate, focal, minimal
	33694-2	Mononuclear inflammatory cell infiltrate, focal, minimal
83F00130	33702-1	Essentially normal skin
	33702-2	Essentially normal skin
83F00133	33705-1	Essentially normal skin
	33705-2	Essentially normal skin

GLP Study 82-038

Table II

Acute Dermal Toxicity (Limit Test) of DMSO Recycle Solvent (TP013), Virgin DMSO (TP014) and DMSO Evaporator Sludge (TP015) in Female New Zealand White Rabbits

Group 1 - 2 ml/kg TP013

83F00335	33971-1	Epidermal hyperplasia, focal, mild Purulent exudate, surface keratin, focal, mild Mononuclear inflammatory cell infiltrate, focally extensive, mild
	33971-2	Epidermal hyperplasia, diffuse, moderate Purulent exdudate, surface keratin, multifocal, mild-moderate Mononuclear inflammatory cell infiltrate, focally extensive, mild-moderate
83F00339	33975-1	Epidermal hyperplasia, multifocal, mild Purulent exudate, surface keratin, multifocal, mild Mononuclear and heterophilic inflammatory cell infiltrate, multifocal, mild-moderate
	33975-2	Epidermal hyperplasia, focal, moderate Purulent exudate, surface keratin, focal, mild Mononuclear and heterophilic inflammatory cell infiltrate, focal, mild-moderate
83F00342	33978-1	Essentially normal skin
	33978-2	Essentially normal skin
83F00347	33982-1	Mononuclear inflammatory cell infiltrate, focal, minimal
	33982-2	Mononuclear and heterophilic inflammatory cell infiltrate, focal, mild
83F00356	33990-1	Mononuclear inflmmatory cell infiltrate, focal, minimal
	33990-2	Essentially normal skin

Group 2 - 2 ml/kg TP014

83F00337	33973-1	Mononuclear inflammatory cell infiltrate, multifocal, minimal
	33973-2	Mononuclear inflammatory cell infiltrate, focal, minimal
83F00343	33979-1	Mononuclear inflammatory cell infiltrate, focal, minimal
	33979-2	Mononuclear inflammatory cell infiltrate, focal, minimal
83F00349	33984-1	Essentially normal skin
	33984-2	Essentially normal skin

83F00353	33987-1	Mononuclear inflammatory cell infiltrate, focal, mild
	33987-2	Mononuclear inflammatory cell infiltrate, focal, minimal, with cross section of hair shaft in center
83F00354	33988-1	Mononuclear inflammatory cell infiltrate, diffuse, minimal
	33988-2	Essentially normal skin

Group 3 - 2 ml/kg TP015

83F00336	33972-1	Essentially normal skin
	33972-2	Essentially normal skin
83F00341	33977-1	Essentially normal skin
	33977-2	Mononuclear inflammatory cell infiltrate, multifocal, minimal
83F00345	33980-1	Essentially normal skin
	33980-2	Mononuclear inflammatory cell infiltrate, multifocal, minimal
83F00348	33983-1	Essentially normal skin
	33983-2	Mononuclear inflammatory cell infiltrate, multifocal, minimal
83F00351	33985-1	Mononuclear inflammatory cell infiltrate, focal, minimal
	33985-2	Essentially normal skin

Group 4 - Saline Control

83F00338	33974-1	Essentially normal skin
	33974-2	Mononuclear inflammatory cell infiltrate, diffuse, minimal
83F00340	33976-1	Mononuclear inflammatory cell infiltrate, focal, minimal
	33976-2	Mononuclear inflammatory cell infiltrate, multifocal, minimal
83F00346	33981-1	Essentially normal skin
	33981-2	Mononuclear inflammatory cell infiltrate, multifocal, minimal
83F00352	33986-1	Essentially normal skin
	33986-2	Essentially normal skin
83F00355	33989-1	Mononuclear inflammatory cell infiltrate, multifocal, minimal
	33989-2	Mononuclear inflammatory cell infiltrate, multifocal, minimal

GLP Study 82038

Table III

Acute Dermal Toxicity (Limit Test) of DMSO Recycle Solvent (TP013), Virgin DMSO (TP014) and DMSO Evaporator Sludge (TP015) in Male and Female New Zealand White Rabbits

Incidence of Microscopic Skin Lesions by Sex and Experimental Group

Group#	Sex	Dosage	Normal Skin	Infiltration	Purulent Exudate	Hyperplasia
1	M	TP013 2 ml/kg	2/5	3/5	0/5	0/5
2	M	TP014 2 ml/kg	3/5	2/5	0/5	1/5
3	M	TP015 2 ml/kg	1/5	4/5	0/5	0/5
4	M	Control	3/5	2/5	0/5	0/5
1	F	TP013 2 ml/kg	1/5	4/5	2/5	2/5
2	F	TP014 2 ml/kg	1/5	4/5	0/5	0/5
3	F	TP015 2 ml/kg	1/5	4/5	0/5	0/5
4	F	Control	1/5	4/5	0/5	0/5

OFFICIAL DISTRIBUTION LIST

Commander
US Army Medical Research
and Development Command
ATTN: SGRD-RMS/Mrs. Madigan
Fort Detrick, Frederick MD 21701

Defense Technical Information Center
ATTN: DTIC-DDA (12 copies)
Cameron Station
Alexandria VA 22314

Director of Defense Research and Engineering
ATTN: Assistant Director, Environmental
and Life Sciences
Washington DC 20301

The Surgeon General
ATTN: DASG-TLO
Washington DC 20314

HQ DA (DASG-ZXA)
WASH DC 20310

Commandant
Academy of Health Sciences
ATTN: HSHA-CDM
Fort Sam Houston TX 78234

Assistant Dean
Institute and Research Support
Uniformed Services University
of Health Sciences
6917 Arlington Road
Bethesda MD 20014

Commander
US Army Environmental Hygiene Agency
Aberdeen Proving Ground MD 21070

US Army Research Office
ATTN: Chemical and Biological Sciences
Division
P.O. Box 1221
Research Triangle Park NC 27709

Biological Sciences Division
Office of Naval Research
Arlington VA 22217

Director of Life Sciences
USAF Office of Scientific Research (AFSC)
Bolling AFB
Washington DC 20332

Director
Walter Reed Army Institute of Research
Washington DC 20307

Commander
US Army Medical Research Institute
of Infectious Diseases
Fort Detrick, Frederick MD 21701

Commander
US Army Research Institute
of Environmental Medicine
Natick MA 01760

Commander
US Army Institute of Surgical Research
Brooke Army Medical Center
Fort Sam Houston TX 78234

Commander
US Army Medical Bioengineering
Research and Development Laboratory
Fort Detrick, Frederick MD 21701

Commander
US Army Aeromedical Research Laboratory
Fort Rucker AL 36362

Commander
US Army Research Institute
of Chemical Defense
Aberdeen Proving Ground
Edgewood Arsenal MD 21010

Commander
Naval Medical Research Institute
National Naval Medical Center
Bethesda MD 20014

Commander
USAF School of Aerospace Medicine
Aerospace Medical Division
Brooks Air Force Base TX 78235